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Experimental characterization of methanol-acetic acid fixative sessile drop dynamics in dry and humid air by video imaging and interference analysis

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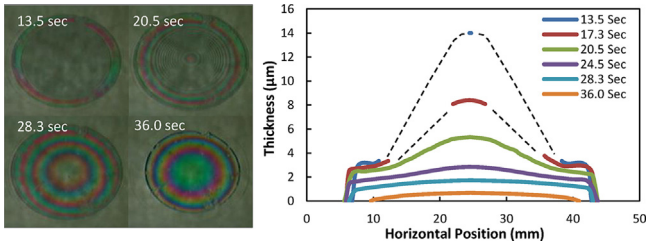
HIGHLIGHTS

- Methanol-acetic acid fixative sessile drop dynamics is characterized.
- A simple setup is built to generate and record interference fringes of the drop.
- Drop shape and surface thinning speed are constructed by interference analysis.
- Different evaporation regimes are suggested by surface thinning speed.

GRAPHICAL ABSTRACT



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ABSTRACT

Dynamics of methanol and acetic acid (3:1, v/v) fixative sessile drop is important for metaphase chromosomal spreads in cytogenetic assays. However, it has not been well characterized by biologists from a physical science point of view. In this work, a simple optical setup was built to record the fixative drop spreading and evaporation process. Drop film thickness, cross-sectional profile and surface thinning speed were constructed from the observed interference patterns to show evolution of the process in both dry and humid air. Surface thinning speed analysis at the drop center suggested different evaporation regimes. The ability of characterizing fluid behavior at a scale comparable to the size of cells by interference fringes is expected to facilitate further understanding of the metaphase spreading process at micro- and nano-scale.

1. Introduction

Chromosome metaphase spread is an important preparation used in both research and clinical laboratories for cytogenetic analyses of cells for chromosome abnormalities [1–5]. It is done by dropping fixative solution (a mixture of methanol and acetic acid with 3:1, v/v, ratio) containing target cells onto a substrate, and let the solution spread out and evaporate. Despite the simplicity of the dropping procedure, achieving a high quality chromosome spread is still an "art" with varying results between laboratories and individuals, limiting the full potential of cytogenetic techniques including their automation for broader applications.

It has been reported that many factors affect the chromosome spreading. Spurbeck et al. used an environmental chamber and found optimal temperature and relative humidity (RH) for chromosome spread [6]. Others used water bath moisture, cooled substrate slide, and elevated temperature or even flame to dry the slide for

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good spread [7–9]. Some reported that a thin water layer on the slide [9,10], certain drop height and substrate slide angle [11], or increased acetic acid fraction would improve chromosome spreading [7], but others reported no or minimal effects of these conditions [8]. It is clear that all the reported factors affect the dynamics of the sessile fixative drop spreading and evaporation, which is critical to the chromosome spreading process. However, such dynamic process was only speculated by biologist, and has not yet been characterized using physical science techniques and at a scale relevant to the cell and chromosome spreading dimensions. Several groups also observed the chromosome spreading process using in situ phase contrast microscopy and identified the importance of water for cell swelling and chromosome stretching [12]; timing and duration of the spreading were also described [8,13]. Nonetheless, these conditions were not linked to the local fluid environment for a better understanding of the process, due again to the lack of drop dynamics information.

To characterize behaviors of sessile drops on solid substrates, gravimetric or optical techniques can be used. Gravimetric analysis can deduce total evaporation rate [14], but lacks size, shape and contact angle information. Several optical techniques have been used to record the size, shape, contact angle of sessile drops. For initial impact and deposition of sessile drops, high speed cameras were used [15,16]. In the case of drops that did not spread very thin, side- and top-view profiles or silhouettes of the drops were recorded by digital cameras [17–19]. An optical setup treating the drop as convex lens was also reported to characterize profile of the drops with low contact angle [20]. For thin film fluids, interference fringes can form. Interference fringes have been used to analyze the edges and the very end of life of low contact angle drops [18,21]. They were also observed in small drops (~ mm in diameter) with stronger spreading and lower surface slope [22,23], but had not been used for measuring drop dynamics in details.

In this paper, we used video imaging and interference fringe analysis to characterize the behaviors of methanol-acetic acid (3:1, v/v) fixative sessile drops without involving cells. Due to significant spreading of the methanol-acetic acid liquid system, the drops spread completely with diameters over 37 mm; interference fringes showed up soon (less than 10 s) after drop deposition, then covered the whole drop area and stayed for a large portion of the drop lifetime. A simple optical setup was built to record drop images and interference fringes to extract diameter, lifetime, dynamic drop profile, thickness and surface thinning speed information. Different evaporation regimes were suggested at the drop center by surface thinning speed analysis for both dry and humid air. This work serves as the first step of interrogating the effect of fixative fluid local environment on chromosome spreading at micro- and nano-scale.

2. Materials and methods

2.1. Materials

Methanol and acetic acid were purchased from Sigma-Aldrich with HPCL grade (≥99.9%) and ACS reagent grade (≥99.7%) respectively. Fixative solution was prepared fresh before the experiments by mixing methanol and acetic acid at 3:1, v/v ratio and stored in a glass vial. A manual pipette was used to dispense 10 μl of fixative solution onto a substrate slide from a fixed position with pipette tip within 5 mm from the slide surface.

The substrate slides used in our experiment were Si substrates cut into uniform width from a 4″ diameter Si wafer with a thickness ~500 μm. The substrate slides were cleaned by RCA step 1 clean (water, ammonium hydroxide, hydrogen peroxide mixture with 5:1:1 ratio at 75 °C for 15 min) to render the surface hydrophilic, and then stored at room temperature until use.

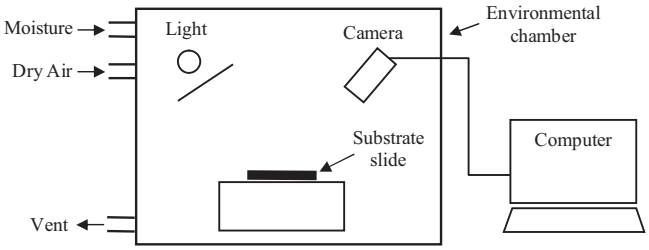

Fig. 1. Schematic diagram of the experimental setup (see text for details).

2.2. Methods

Fig. 1 shows a schematic diagram of the optical setup. The setup was hosted inside an ETS environmental chamber (Electro-Tech Systems, Inc., Glenside, PA) with approximately 0.368 m^3 usable space. Fluorescent lamp of the chamber was used as the light source to illuminate the fixative drop on substrate slide. A clean room wipe was used in front of the lamp as a diffuser to uniform the illumination. A Q-See color camera (Anaheim, CA) oriented 30° from the substrate slide normal, and a computer with a video card and WinTV 2000 software (Hauppauge Computer Works, Inc., Hauppauge, NY) were used to capture the experiments. Videos were saved as AVI files with RGB color at 30 fps. Substrate slides were placed on top of a hotplate. A ruler was taped on the hotplate surface as a scale.

Diameter and lifetime of the drops were measured from the videos by ImageJ software (National Institute of Health). ImageJ was also used to split the video into Red/Green/Blue channels. Interference fringes from the green channel were used for data extraction. Similar results were obtained when other channels were used. Assuming a wavelength of 540 nm for the green channel, each order of constructive interference fringe stood for a film thickness of 215.3 nm (see Appendix A: interference fringes and film thickness). To find out how the liquid film thickness h changed with time at any position within the drop, light intensity at that position over time was plotted using ImageJ's "Plot Z-axis Profile" command in the menu. Interference maxima and the orders of constructive interference were identified, which were converted to the corresponding film thickness. To obtain the surface thinning speed V_s (i.e. $\partial h/\partial t$, t is the time) at that position, the thickness data were fitted by polynomial using Matlab™. V_s was calculated as the first derivative of the fitted curve. To construct cross-sectional profile of the drop at certain time, light intensity of the drop was plotted along a horizontal line that passed the drop center and crossed the whole drop. Interference extrema were identified, and their orders and corresponding film thicknesses were obtained by correlating with the temporal intensity history of the drop.

The environmental chamber was placed under a chemical fume hood. The temperature of the chamber was controlled by the building air conditioning system, and was 23.4 ± 0.6 °C during our experiments. Relative humidity (RH) of the chamber was adjusted by either purging the chamber with building compressed dry air, or by an ultrasonic humidifier to supply moisture to the chamber. For dry air experiments, the RH was reduced to <0.8% by compressed dry air before fixative dropping. The compressed dry air was turned off during the experiment and the RH was below 1.5% throughout the experiments. For all the experiments, a built-in fan was used to circulate the air inside the chamber. The fan was turned off during fixative spreading.

3. Results and discussions

Spreading and evaporation of liquids on solid substrates is encountered in many practical processes and has been studied